Isolation and Identification of Two Serotypes of Broad Bean Wilt Virus

J. K. Uyemoto and R. Provvidenti

Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva 14456.

Approved by the Director as Journal Paper No. 2091.

The authors are grateful to R. J. Shepherd and R. Hull for their suggestions.

ABSTRACT

Based upon spur formation in agarose gel plates, seven isolates of broad bean wilt virus (BBWV) were divided into two distinct serological types (serotypes). Group I included isolates from pea, spinach, broad bean, nasturtium, and *Plantago* I, whereas isolates from lettuce and *P.* II were members of the second group.

Phytopathology 64:1547-1548

Additional key words: BBWV serotypes.

Broad bean wilt virus (BBWV) is worldwide in occurrence, and reportedly has been isolated from a number of weeds and cultivated plants (8). In New York State the virus has been found to infect pea (*Pisum sativum* L.) (4), spinach (*Spinacia oleracea* L.) (3), lettuce (*Lactuca sativa* L.), pigweed (*Amaranthus retroflexus* L.), and sowthistle [*Sonchus asper* (L.) Hill] (1). During routine indexing of certain weeds, particularly *Plantago lanceolata* L., using as indicator host *Chenopodium quinoa* Willd., we obtained several virus isolates which on diagnostic species incited symptoms similar to those caused by broad bean wilt virus (BBWV) (3, 8). Symptomatologically, the pea, spinach, lettuce, *Plantago* I and II virus isolates could not be differentiated by host-range reaction (R. Provvidenti, *unpublished*). Serological tests confirmed their identity, but a difference in antigenicity among the many isolates was evident.

The two serological types (serotypes) from *P. lanceolata,* Pl-I and Pl-II, were compared serologically with known cultures of BBWV which included nasturtium ringspot (ATCC PV-176) (6), and isolates from pea (PO) (4), spinach (ATCC PV-132) (3), lettuce (ATCC PV-131) (1), and broad bean (Australian isolate supplied by R. J. Shepherd) (7).

Although *C. quinoa* proved to be an extremely sensitive indicator host for BBWV, the virus was maintained and increased in pea (*Pisum sativum* 'Bonneville'). Virus isolates Pl-I and Pl-II were selected for increase and purification. Infected pea tissue was homogenized in a Waring Blendor with potassium phosphate buffer (0.1M, pH 7.6) containing 0.1% thioglycollic acid. The homogenate was squeezed through cheesecloth and refrigerated overnight with 10% butanol. Following two alternating low- and high-speed cycles of centrifugation (Sorvall GSA rotor, 8,000 rpm/10 min; Spinco 30 rotor, 28,000 rpm/2 h), the final high speed pellets were dispersed in potassium phosphate buffer, 0.037M, pH 7.6, and subjected to zone electrophoresis (9). Final preparations were highly infectious. Rabbits were immunized with a series of subcutaneous (antigen emulsified with Freund's incomplete adjuvant) and intravenous injections and bled 7 days after the last injection.

Serological tests were conducted in 1% agarose gel plates (Mann Research Laboratories, Inc.) using an eight-member well pattern surrounding a center antiserum depot. Expressed leaf sap of infected pea plants were sources of virus antigens. Antibody titers for antisera type I and II were 128 and 16 (reciprocal of dilution); 128 and 64, respectively, for homologous and heterologous virus antigens. When the virus isolates and type I antiserum were compared in a common plate, a single congruent line formed against antigen wells containing isolates nasturtium ringspot, broad bean, PO, spinach, and Pl-I. This precipitin line extended over the lines produced by Pl-II and lettuce isolates, which were identical. There was no visible reaction to the healthy pea control (Fig. 1). Reciprocal tests with type II antiserum produced a single continuous line between isolates Pl-II and lettuce that spurred over the heterologous virus (type I) line. A BBWV antiserum kindly supplied by P. R. Smith (Victorian Plant Research Institute, Burnley, Australia) reacted similarly to type I antiserum with type I isolates, but failed to produce a visible line with type II isolates. It is uncertain as to whether the latter serum source was a primary or long-term antiserum (5). However, based on the strength of the precipitin line to type I isolates, we do not feel that a third serotype was present among the isolates tested.

Previous reports indicate a single antigenic entity among the various isolates of BBWV (2, 3, 7, 8). Our test suggests that there exist at least two serologically distinct strains of the virus. It is proposed that those isolates which react identically, in agarose gel double-diffusion

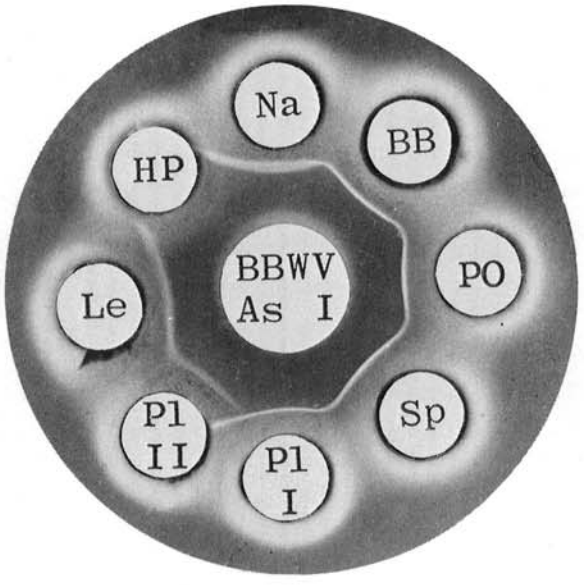

Fig. 1. Agarose-gel-diffusion plate of broad bean wilt virus (BBWV) antiserum, type I (center well) using infected pea sap containing virus isolates nasturtium ringspot (Na), broad bean wilt (BB), pea (PO), spinach (Sp), *Plantago* I (Pl-I), *Plantago* II (Pl-II), lettuce (Le), and healthy pea (HP) deposited in the peripheral wells.

tests, with Pl-I or II be designated serotype I or II, respectively. The term serotype has been defined earlier (10).

LITERATURE CITED

1. ATILANO, R. A. 1971. Identification of three viruses from New York lettuce growing in organic soil. M. Sc. Thesis, Cornell University, Ithaca, N.Y. 69 p.
2. FROWD, J. A., and J. A. TOMLINSON. 1972. Relationships between a parsley virus, nasturtium ringspot virus and broad bean wilt virus. Ann. Appl. Biol. 72:189-195.
3. SCHROEDER, W. T., and R. PROVVIDENTI. 1970. A destructive blight of Spinacea oleracea incited by a strain of broad bean wilt virus. Phytopathology 60:1405-1406.
4. SCHROEDER, W. T., R. PROVVIDENTI, and F. L. MC EWEN. 1960. An unusual virus isolated from Pisum sativum affected by streak. Phytopathology 50:654 (Abstr.).
5. SHEPARD, J. F., G. A. SECOR, and D. E. PURCIFULL. 1974. Immunochemical cross-reactivity between the dissociated capsid proteins of PVY group plant viruses. Virology 58:464-475.
6. SMITH, K. M. 1950. Some new virus diseases of ornamental plants. J. Roy. Hortic. Soc. 75:350-353.
7. TAYLOR, R. H., P. R. SMITH, C. REINGANUM, and A. J. GIBBS. 1968. Purification and properties of broad bean wilt virus. Austr. J. Biol. Sci. 21:929-935.
8. TAYLOR, R. H., and L. L. STUBBS. 1972. Broad bean wilt virus. No. 81 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, Kent. 4 p.
9. UYEMOTO, J. K., and R. G. GROGAN. 1969. Chemical characterization of tobacco necrosis and satellite viruses. Virology 39:79-89.
10. UYEMOTO, J. K., R. G. GROGAN, and J. R. WAKEMAN. 1968. Selective activation of satellite virus strains by strains of tobacco necrosis virus. Virology 34:410-418.